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WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

#6

10/039,383

(51) International Patent Classification 6 : <b>A61K 39/39, 39/245</b>		<b>A1</b>	(11) International Publication Number: <b>WO 95/30437</b>
			(43) International Publication Date: 16 November 1995 (16.11.95)
(21) International Application Number: <b>PCT/US95/06053</b>		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).	
(22) International Filing Date: <b>8 May 1995 (08.05.95)</b>			
(30) Priority Data: 240,373 10 May 1994 (10.05.94) <b>US</b>			
(60) Parent Application or Grant (63) Related by Continuation <b>US</b> 240,373 (CON) Filed on 10 May 1994 (10.05.94)		<b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(71) Applicant (for all designated States except US): <b>AMERICAN HOME PRODUCTS CORPORATION [US/US]; Five Giralda Farms, Madison, NJ 07940 (US).</b>			
(72) Inventor; and (75) Inventor/Applicant (for US only): <b>CHU, Hsien-Jue [US/US]; 1706 13th Avenue, Fort Dodge, IA 50501 (US).</b>			
(74) Agents: <b>SCHAFER, Robert et al.; Darby &amp; Darby, P.C., 805 Third Avenue, New York, NY 10022 (US).</b>			
(54) Title: <b>IMPROVED MODIFIED LIVE BRSV VACCINE</b>			
(57) Abstract <p>The invention provides an improved BRSV vaccine composition, which advantageously provides immunity from infection after a single administration. The composition comprises a modified live BRS virus and an adjuvant, which in combination provide immunity from BRSV infection after a single administration, and elicit an immune response specific to BRSV and including cell-mediated immunity and local (secretory IgA) immunity. In a preferred embodiment, the BRS virus is strain 375, and the adjuvant comprises an unsaturated turpin hydrocarbon, preferably squalene or squalane, and a polyoxypropylene-polyoxyethylene block copolymer, most preferably one where the copolymer has a polyoxypropylene (POP) component with an average molecular weight of about 3250 to 4000 and the polyoxyethylene (POE) component comprises about 10-20 % of the total molecule. The adjuvant may optionally include a surfactant, preferably a polyoxyethylenesorbitan monooleate.</p>			

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### IMPROVED MODIFIED LIVE BRSV VACCINE

#### Field of the Invention

This invention pertains to improved methods for inducing protective immunity against Bovine Respiratory Syncytial Virus (BRSV), specifically employing a modified live vaccine suitable for administration to recipient animals in a single dose.

#### Background to the Invention

Bovine Respiratory Syncytial Virus (BRSV) is now recognized as an important etiologic agent in the Bovine Respiratory Disease Complex (BRDC). Disease is characterized by rapid breathing, coughing, loss of appetite, ocular and nasal discharge, and elevated temperatures. In an acute outbreak, death may follow within 48 hours of onset of symptoms.

BRSV infects cattle of all ages, including nursing calves. BRSV is considered the most common viral pathogen in enzootic pneumonia in calves, and has also been associated with pulmonary emphysema among newly weaned calves. Thus, there is a need for effective prophylaxis against this virus in cattle and dairy herds.

Establishing protective immunity against BRSV is problematic. As in some other virally-mediated diseases, the levels of serum antibodies against BRSV do not necessarily correlate with protection against disease. This phenomenon may reflect a role for locally produced IgA directed against BRSV (Kimmman et al., J. Clin. Microbiol. 25: 1097-1106, 1987), and/or a requirement for cell-mediated immunity to mount an effective defense against this virus. Establishing protective immunity

in nursing calves presents additional obstacles, since maternal antibodies to BRSV may deplete the injected immunogen and effectively neutralize the vaccine. Finally, the inconvenience and expense of multiple vaccinations makes a single-dose vaccine desirable. Thus there is a need in the art for one-dose BRSV vaccine formulations that elicit a vigorous and multi-faceted immune response.

The standard administration regimen for prior art BRSV vaccines is two doses (Stott et al., J. Hyg.Camb. 93: 251-261, 1984; Thomas et al., Agri-Practice 5: 1986; and Syvrud et al., Vet.Med. 83: 429-430, 1988; Veterinary Pharmaceuticals & Biologicals, Edition 8 1993/94, pp. 484, 740-741, 956-960, 982-983.) As shown in Kucera et al. (Agri-Practice, Vet.Med., Vol. 78, October 1983, pp. 1599-1604, 1983), a single experimental BRSV vaccination induced relatively low levels of serum neutralization (SN) antibody titer to BRSV, whereas two doses of the vaccine elicited 1:10 to 1:320 SN antibody titers. Furthermore, in herds apparently exposed to BRSV during field trials, approximately 48% of non-vaccinated animals required treatment for respiratory disease, compared with 27% and 21% among single-dose and double-dose vaccinates, respectively. However, the causative agent for respiratory disease in the field trials was not conclusively shown to be BRSV. Also, it was noted that a single-dose vaccine did not appear to be very immunogenic. Later evaluations concluded that two doses of this vaccine would be essential to obtain good protection (Bovine Vet.Forum 1: No.2 pp. 2-16, 1986; Syvrud, et al., Vet.Med. 429-430, 1988).

European Patent Application No. 129,923 (published February 1, 1985 and issued as a patent July 9, 1988) describes a method of preparing a live BRSV vaccine that involves dissolving the live vaccine in an inactivated vaccine containing one or more antigens (particularly inactivated influenza virus) formulated as an oil-in-water emulsion. A serological response was obtained in young animals still having maternal immunity. The application also describes a modified-live preparation including BRSV and adjuvant. However, no data

were presented on the protective efficacy of any BRSV vaccine against BRSV challenge.

One object of the invention is to provide an effective vaccine against BRSV that elicits protective immunity and prevents disease caused by this virus.

A further object of the invention is to provide an adjuvant suitable for use in a BRSV vaccine, wherein the adjuvant enhances the immunogenicity of the virus so as to elicit protective immunity after a single dose of the vaccine.

10

#### Summary of the Invention

The invention encompasses a composition for enhancing immune responses comprising a block copolymer, such as a polyoxypropylene-polyoxyethylene (POP-POE) block copolymer, preferably Pluronic® L121 (e.g. U.S. Patent 4,772,466), and an organic component, such as a metabolizable oil, e.g. an unsaturated turpin hydrocarbon, preferably squalane (2,6,10,15,19,23-hexamethyltetracosane) or squalene. The composition may also include a non-ionic detergent or surfactant, preferably a polyoxyethylene sorbitan monooleate such as a Tween® detergent, most preferably Tween®-80, i.e. polyoxyethylene (20) sorbitan monooleate.

In this stock adjuvant mixture, the block copolymer, organic oil, and surfactant may be present in amounts ranging from about 10 to about 40 ml/L, about 20 to about 80 ml/L, and about 1.5 to about 6.5 ml/L, respectively. In a preferred embodiment of the stock adjuvant, the organic component is squalane present in an amount of about 40 mL/L, the surfactant is polyoxyethylenesorbitan monooleate (Tween®-80) present in an amount of about 3.2 ml/L, and the POP-POE block copolymer is Pluronic® L121 present in an amount of about 20 ml/L. Pluronic® L121 is a liquid copolymer at 15-40 C, where the polyoxypropylene (POP) component has a molecular weight of 3250 to 4000 and the polyoxyethylene (POE) component comprises about 10-20%, preferably 10%, of the total molecule.

In another aspect, the present invention provides an immunogenic composition for immunizing an animal against infection by Bovine Respiratory Syncytial Virus (BRSV),

comprising a modified live BRS Virus combined with the above adjuvant and a pharmaceutically acceptable stabilizer, carrier or diluent. The adjuvant is present in this vaccine composition at a final concentration of about 1-25% (v/v),  
5 preferably 5% (v/v). The composition may also include other viruses, such as Infectious Bovine Rhinotracheitis Virus (IBRV), Bovine Viral Diarrhea (BVDV), and Parainfluenza 3 (PI-3V), and may be administered by intramuscular or subcutaneous routes.

10 In still another aspect, the present invention provides a method for protecting an animal against disease caused by Bovine Respiratory Syncytial Virus, by administering a single dose of the above vaccine comprising modified-live BRSV and adjuvant.

15

#### Detailed Description of the Invention

All patents, patent applications, and other literature cited herein are hereby incorporated by reference in their entirety. In the case of inconsistencies, the present  
20 disclosure will prevail.

As used herein, a "modified live vaccine" is a vaccine comprising a virus that has been altered, typically by passaging in tissue culture cells, to attenuate its ability to cause disease, but which maintains its ability to protect  
25 against disease or infection when administered to animals.

"Adjuvant" means a composition comprised of one or more substances that enhances the immunogenicity and efficacy of BRSV when combined with BRSV in a vaccine composition.

An "infectious unit" of BRSV is defined as a TCID<sub>50</sub>,  
30 or the amount of virus required for infecting or killing 50% of tissue culture cells.

The present invention provides a vaccine against BRSV that is suitable for single-dose administration. The vaccine is of the modified live virus variety. This provides the  
35 advantage of preserving the immunogenicity and/or efficacy of the virus while reducing its virulence.

The vaccine may be prepared from freshly harvested viral cultures by methods that are standard in the art (see

Example 1 below.) That is, the virus may be propagated in tissue culture cells such as human diploid fibroblasts or preferably MDBK (Madin-Darby Bovine Kidney) or other bovine cells. The growth of the virus is monitored by standard techniques (observation of cytopathic effect, immunofluorescence or other antibody-based assays), and harvested when a sufficiently high viral titer has been achieved. The viral stocks may be further concentrated or lyophilized by conventional methods before inclusion in the vaccine formulation. Other methods, such as those in described in Thomas, et al., Agri-Practice, V.7 No. 5, pp.26-30., can be employed.

The vaccine of the present invention comprises the modified live virus combined with one or more pharmaceutically acceptable stabilizers, carriers and adjuvants. Carriers suitable for use include saline, phosphate-buffered saline, Minimal essential media (MEM), or MEM with HEPES buffer. Stabilizers include but are not limited to sucrose, gelatin, peptone, digested protein extracts, such as NZ-Amine or NZ-Amine AS. In particular, the present invention includes an adjuvant that enhances the immunogenicity of the modified live virus and provides for a single administration to elicit protective immunity.

Non-limiting examples of suitable adjuvants include squalane and squalene (or other oils of animal origin); block copolymers such as Pluronic® (L121) Saponin; detergents such as Tween®-80; Quil® A, mineral oils such as Drakeol® or Marcol®, vegetable oils such as peanut oil; Corynebacterium-derived adjuvants such as corynebacterium parvum; Propionibacterium-derived adjuvants such as Propionibacterium acne; Mycobacterium bovis (Bacillus Calmette and Guérin, or BCG); interleukins such as interleukin 2 and interleukin-12; monokines such as interleukin 1; tumor necrosis factor; interferons such as gamma interferon; combinations such as saponin-aluminum hydroxide or Quil®-A aluminum hydroxide; liposomes; iscom adjuvant; mycobacterial cell wall extract; synthetic glycopeptides such as muramyl dipeptides or other derivatives; Avridine; Lipid A; dextran sulfate; DEAE-Dextran

D) Harvesting of Virus: Culture fluids were harvested into sterile vessels. Multiple harvests may begin when 50% of the cell sheet displays characteristic cytopathology, and continue until 100% of the cells are affected. The virus fluids may or may not be clarified by centrifugation or filtration. Viral fluids are stored at -50 C or colder, or are lyophilized and stored at 2 to 8 C.

For preparation of a final vaccine, viral stocks, either alone or in combination, are mixed with adjuvant.

When liquid viral stocks are used, 19 parts viral stock are mixed with one part adjuvant, preferably the adjuvant of Example 2. When lyophilized viral stocks are used, a dilute 5% (v/v) solution of adjuvant in saline is prepared (mix 1 part adjuvant with 19 parts saline). The lyophilized viral stock is reconstituted (rehydrated) with the diluted adjuvant to form the final vaccine composition. Thimerisol may be added to the final formulation, to a final concentration of 1:10,000.

Example 2: Formulation of a preferred stock adjuvant

A preferred adjuvant for use in the present invention was prepared according to the following formulation:

polyoxypropylene-polyoxyethylene block copolymer (e.g. Pluronic® L121, BASF, Parsippany, NJ)	20 ml
Squalane (e.g., Kodak, Rochester, NY)	40 ml
polyoxyethylenesorbitan monooleate (e.g. Tween®-80, Sigma Chemical, St. Louis, MO)	2 ml
buffered salt solution (e.g. D-V PAS Solution, Ca, Mg Free)	936.8 ml

The ingredients are mixed and homogenized until a stable mass or emulsion is formed. Prior to homogenization, the ingredients or mixture can be autoclaved. The emulsion may be further sterilized by filtration. Formalin may be added up to a final concentration of 0.2%. Thimerosal may be added to a final dilution of 1:10,000.



**Example 3: Enhancement of a Modified Live BRSV Vaccine**

For this study, two BRSV vaccines were prepared, one with and one without the adjuvant mixture described in Example 2. The vaccine lacking adjuvant contained 2.52 log infectious units of BRSV per 2 ml, while the vaccine containing adjuvant contained 2.96 log infectious units per 2 ml and 5% (v/v) adjuvant.

Each of twenty cattle received a 2-ml dose of vaccine lacking adjuvant, ten intramuscularly and ten subcutaneously. Five additional cattle received a 2-ml dose of vaccine containing adjuvant. All vaccinations were repeated at 21 days. Serum samples were obtained on the sixth day following the second vaccination, and were tested for the presence of anti-BRSV serum neutralization antibodies. The serum neutralization antibody assay is described in Example 4

The results of this study indicated that 4 of the 5 calves inoculated with the adjuvant-containing BRSV vaccine showed evidence of anti-BRSV antibodies (seroconversion), while none of the twenty animals inoculated with adjuvant-lacking BRSV vaccine showed evidence of antibodies. This indicates that the adjuvant described in Example 2 has the property of enhancing the immunogenicity of modified live BRSV vaccines.

**Example 4: Single-dose Administration of Improved BRSV vaccine**

The following vaccination and challenge study was performed in order to determine whether a single immunization modified-live Bovine Respiratory Syncytial Virus (BRSV) formulated with an adjuvant would induce protective immunity in cattle. Secondly, the study was designed to determine whether concurrent administration of modified-live Bovine Viral Diarrhea Virus (BVDV), Bovine Herpesvirus, Type 1 (BHV-1 or IBRV), and Bovine Parainfluenza Virus (PI3) would interfere with the induction of protective immunity to BRSV.

A) Experimental Vaccines: Modified-live Bovine Respiratory Syncytial Virus (BRSV) at five passages beyond the master seed was grown on Madin Darby Bovine Kidney (MDBK) cells at master cell stock passage 20. Briefly, MDBK cells were planted in 850 cm<sup>2</sup> roller bottles at a density of  $3 \times 10^7$  cells

per roller bottle in Minimum Essential Media (MEM) containing 5% bovine serum, 0.5% LAH, and 30 µg/mL Gentamycin. Cells were allowed to grow at 37°C for 2 days prior to infection with virus. Media was decanted from the roller bottles and virus added at a Multiplicity of Infection of 1:600 in 100 mL of virus propagation media per bottle (MEM containing 2% bovine serum, 0.5% LAH, and 30 µg/mL Gentamycin). Seven days after infection, 100% cytopathology was present and supernatant fluids were harvested. The virus was stabilized with 25% (v/v) SGGK3 stabilizer and lyophilized. On the day of vaccination, the lyophilized virus was reconstituted with 5% (v/v) adjuvant diluted in saline diluent (See, Example 2). Reconstituted BRSV virus was combined with PI3, BVDV, and BHV-1 viruses. The titer of each component of the vaccine was determined by replicate titration on the day of vaccination.

B) Experimental Animals Used: A total of 30 cattle were used for this study. These cattle were susceptible to BRSV as indicated by a serum neutralizing (SN) antibody titer of <2 on the day of vaccination for test animals and on the day of challenge for controls. Animals were housed outside with access to a three sided shelter, open to the south. Controls were housed separately from vaccinates prior to challenge in order to avoid exposure to vaccine virus. A complete ration was provided once each day, hay and water were supplied ad libitum.

C) Vaccination: A two mL volume of the combination vaccine was administered once to each vaccinate. Twenty (20) animals were vaccinated (ten by subcutaneous route and ten by intramuscular route) and the remaining ten animals were not vaccinated and served as challenge controls.

D) Experimental Challenge: Animals were challenged with virulent BRSV virus fourteen days following the vaccination. A minimum of  $10^{5.7}$  TCID<sub>50</sub> of virulent BRSV virus was administered to each calf by aerosol challenge on three consecutive days.

E) Clinical Observations: Cattle were observed daily from -2 to 14 days following challenge for clinical signs of disease and fever (rectal temperature). Cattle were

observed for signs of BRSV infection including, but not limited to, nasal and ocular discharge, conjunctivitis, coughing, dyspnea, anorexia, and depression. Rectal temperature was recorded daily throughout the observation period.

5 F) Assays:

1. Serum Neutralizing Antibody Assay (SN)

Serial dilutions of heat-inactivated serum were mixed with equal volumes of viral suspensions, in a varying serum-constant virus neutralization test using 100 to 500 TCID<sub>50</sub> of BRSV. The serum virus mixture was incubated at 37°C for 1 hour then inoculated onto VERO cells in 96 well microtiter plates. The presence of SN antibody titers was indicated by the absence of virus as detected by cytopathic effect. For the determination of SN antibody titers, 50% neutralization endpoints were calculated according to the method of Reed and Muench.

2. Titration of Virus in Final Dilution of Vaccine

The BRSV virus titer in the vaccine was determined by replicate titration on the day of vaccination. Briefly, the combination vaccine was combined with appropriate neutralizing antisera. The vaccine and antisera mixture was incubated at 37°C for 45 to 60 minutes. Serial dilutions of the vaccine and antisera were made and inoculated onto VERO cells. The presence of virus was indicated by the presence of cytopathic effect and confirmed by specific immunofluorescence (FA). Virus titer was calculated on each replicate by the method of Reed and Muench. The mean titer of BRSV fraction of the vaccine was 10<sup>3.4</sup> TCID<sub>50</sub> per dose.

3. Titration of Challenge Virus

The dilution of the BRSV challenge virus administered was serially diluted and inoculated onto MDBK cells in 96 well microtiter plates. The presence of virus was indicated by the presence of cytopathic effect and confirmed by specific immunofluorescence as described for virus isolation.

To interpret the results, clinical scores were assigned as follows:

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	Clinical sign	Score/observation
	Nasal Discharge	
	Severe serous	2
	Mild mucopurulent	2
5	Moderate mucopurulent	3
	Severe mucopurulent	4
	Ocular Discharge	
	Severe serous	1
	Mild mucopurulent	2
10	Moderate mucopurulent	3
	Severe mucopurulent	4
	Conjunctivitis	2
	Coughing	2
	Dyspnea	2
15	Anorexia	1
	Hyperemia and reddening of nasal mucosa	1
	Fever (must be at least 1°F above baseline)	
	103.5 to 103.9°F	1
20	104.0 to 104.9°F	2
	105.0 to 105.9°F	3
	≥ 106.0°F	4

Mild serous nasal or ocular discharge was considered to be normal for cattle housed outside. Fever was considered significant only if it was at least one degree above the baseline body temperature. The baseline body temperature was determined as the average body temperature for each animal on the day prior to and day of challenge.

Total clinical scores for each animal were summed. Clinical scores of the vaccinates and controls were compared by Mann Whitney Ranked Sum Analysis.

Clinical signs of disease were observed in the control cattle from days 5 through 10 after challenge (Table 1). All of the controls (100%) were observed to have signs of respiratory disease on multiple days. Specific signs of respiratory disease included severe serous nasal discharge (discharge actually dripping from nostril), mucopurulent nasal discharge, ocular discharge, and coughing. The average clinical score for control calves was 3.7.

By comparison, respiratory signs were much less prevalent in vaccinated animals. Only 40% of the vaccinates had any signs of respiratory disease and only two (10%) had clinical signs on multiple days. The average clinical score for the vaccinated group was 1.0. There was a statistically significant reduction in clinical disease in the vaccinates

compared to the controls by Mann Whitney Ranked Sum Analysis (p<0.05).

These data show that a single-dose administration of adjuvanted modified-live BRSV virus vaccine, according to the invention, provides protection against virulent BRSV challenge. This vaccine and method is effective, even when other vaccines are coadministered with the BRSV vaccine.

Thus, the invention provides a vaccine composition for immunizing an animal against infection by Bovine Respiratory Syncytial Virus (BRSV). The vaccine comprises a modified live BRS Virus, an adjuvant, and a pharmaceutically acceptable carrier, such that the combination provides immunity from BRSV infection after a single administration, and elicits an immune response specific to BRSV and selected from cell-mediated immunity and local (secretory IgA) immunity.

Cell mediated immunity includes the stimulation of T-Helper Cells, T-Killer Cells, and T-Delayed Hypersensitivity Cells, as well as stimulation of macrophage, monocyte, and other lymphokine and interferon production. The presence of cell mediated immunity can be determined by conventional in vitro and in vivo assays. Local immunity, such as secretory IgA, can be determined by conventional ELISA or IFA assays showing a serum neutralizing antibody titer of 1-2 or greater. According to the invention, the cell mediated or local immunity of consequences is specific to or associated with BRSV.

Claims:

- 1                    1.            A vaccine composition for immunizing an  
1 animal against infection by Bovine Respiratory Syncytial Virus  
2 (BRSV) comprising  
3                    a modified live BRS Virus, an adjuvant, and a  
4 pharmaceutically acceptable carrier, such that the composition  
5 provides immunity from BRSV infection after a single  
6 administration, and elicits an immune response specific to BRSV  
7 and selected from cell-mediated immunity and local (secretory  
8 IgA) immunity.
- 1                    2.            A composition of claim 1, wherein the  
2 adjuvant comprises a polyoxypropylene-polyoxyethylene block  
3 copolymer and an unsaturated turpin hydrocarbon.
- 1                    3.            A composition of claim 2, wherein the  
2 unsaturated turpin hydrocarbon is one of squalene and squalane,  
3 and the copolymer has a polyoxypropylene component with an  
4 average molecular weight of about 3250 to 4000 and the  
5 polyoxyethylene component comprises about 10-20% of the total  
6 molecule.
- 1                    4.            A composition of claim 2 wherein the copolymer  
2 is present in a final concentration of about 0.01 to 1% (v/v)  
3 and the hydrocarbon component is present in a final  
4 concentration of about 0.02 to 2% (v/v).
- 1                    5.            A composition of claim 2, wherein the adjuvant  
2 additionally comprises a surfactant.
- 1                    6.            A composition of claim 4, wherein the adjuvant  
2 also comprises a surfactant present in a final concentration  
3 of about 0.0015 to 0.20% (v/v).
- 1                    7.            A composition of claim 5 or claim 6, wherein the  
2 surfactant is a polyoxyethylene sorbitan monooleate.

1           8. A composition of claim 4, wherein the  
2 hydrocarbon is one of squalene and squalane, and the copolymer  
3 has a polyoxypropylene component with an average molecular  
4 weight of about 3250 to 4000 and the polyoxyethylene component  
5 comprises about 10-20% of the total molecule.

1           9. The composition of any of claims 1-9, further  
2 comprising Bovine Rhinotracheitis Virus (BHV-IV), Bovine Viral  
3 Diarrhea Virus (BVDV), and Parainfluenza 3 Virus (PI-3V).

1           10. A vaccine composition for immunizing an animal  
2 against infection by Bovine Respiratory Syncytial Virus (BRSV)  
3 comprising

4           a modified live BRS Virus, a pharmaceutically  
5 acceptable carrier, and an adjuvant comprising a  
6 polyoxypropylene-polyoxyethylene block copolymer and an  
7 unsaturated turpin hydrocarbon, such that the composition  
8 provides immunity from BRSV infection after a single  
9 administration.

1           11. A composition of claim 10, wherein the  
2 hydrocarbon is one of squalene and squalane, and the copolymer  
3 has a polyoxypropylene component with an average molecular  
4 weight of about 3250 to 4000 and the polyoxyethylene component  
5 comprises about 10-20% of the total molecule.

1           12. A composition of claim 11 wherein the block  
2 copolymer is present in a final concentration of about 0.01  
3 to 1% (v/v) and the organic component is present in a final  
4 concentration of about 0.02 to 2% (v/v).

1           13. A composition of claim 12, wherein the adjuvant  
2 additionally comprises a surfactant present in a final  
3 concentration of about 0.0015 to 0.20% (v/v).

1           14. A composition of claim 13 wherein the surfactant  
2 is polyoxyethylene sorbitan monooleate.

1           15. The composition of any of claims 10-14, further  
2 comprising at least one modified-live virus selected from the  
3 group consisting of Bovine Rhinotracheitis Virus (BHV-IV),  
4 Bovine Viral Diarrhea Virus (BVDV), and Parainfluenza 3 Virus  
5 (PI-3V).

1           16. A method for protecting an animal against  
2 disease caused by Bovine Respiratory Syncytial Virus (BRSV),  
3 comprising the step of administering to said animal an  
4 immunogenic composition comprising  
5           a modified live BRS Virus, an adjuvant, and a  
6 pharmaceutically acceptable carrier, such that the composition  
7 provides immunity from BRSV infection after a single  
8 administration, and elicits an immune response specific to BRSV  
9 and selected from cell-mediated immunity and local (secretory  
10 IgA) immunity.

1           17. A method of claim 16 wherein said virus is  
2 present in a range from about  $10^3$  to about  $10^6$  infectious units  
3 per milliliter.

1           18. A method of claim 16 wherein said administering  
2 step comprises one of intramuscular, subcutaneous,  
3 intraperitoneal, oral, and intranasal administration.

1           19. A method of claim 16 wherein the adjuvant  
2 comprises a polyoxypropylene-polyoxyethylene block copolymer  
3 in a final concentration of about 0.01 to 1% (v/v) and an  
4 unsaturated turpin hydrocarbon in a final concentration of  
5 about 0.02 to 2% (v/v).

1           20. A method of claim 19, wherein the adjuvant  
2 additionally comprises a non-ionic surfactant in a final  
3 concentration of about 0.0015 to 0.20% (v/v).

1           21. A method of claims 20, wherein the hydrocarbon  
2 is one of squalene and squalane, the surfactant is



3 polyoxyethylenesorbitan monooleate, and the copolymer has a  
4 polyoxypropylene component with an average molecular weight of  
5 about 3250 to 4000 and the polyoxyethylene component comprises  
6 about 10-20% of the total molecule.

1           22. The method of any of claims 16-21, further  
2 comprising the coadministration of Bovine Rhinotracheitis Virus  
3 (BHV-IV), Bovine Viral Diarrhea Virus (BVDV), and Parainfluenza  
4 3 Virus (PI-3V).

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 95/06053

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 A61K39/39 A61K39/245

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 129 923 (DUOHAR INTERNATIONAL RESEARCH B.V.) 2 January 1985 see claim 178; example 5 ---	1, 16, 18
A	VET.IMMUNOL.IMMUNOPATHOL., vol. 22, no. 2, 1989 NL, pages 145-60, T.G.KIMMAN ET AL. 'Priming for Local and Systemic Antibody Memory Responses to Bovine Respiratory Syncytical Virus: Effect of Amount of Virus, Virus Replication, Route of Administration and Maternal Antibodies' --- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

8 September 1995

Date of mailing of the international search report

29.09.95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

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Deffner, C-A

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 95/06053

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J.CLIN.MICROBIOL., vol. 25, no. 6, 1987 pages 1097-1106, T.G.KIMMAN ET AL. 'Local and Systemic Antibody Response to Bovine Respiratory Syncytial Virus Infection and Reinfection in Calves with and without Maternal Antibodies' ---	
A	VET.Q, vol. 13, no. 1, 1991 NL, pages 47-59, J.C.BAKER 'Human and bovine respiratory syncytial virus: immunopathologic mechanisms' ---	
A	SEMIN.IMMUNOL., vol. 2, no. 5, 1990 USA, pages 369-374, A.C.ALLISON ET AL. 'Adjuvant formulations and their mode of action' -----	

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 95/06053

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-129923	02-01-85	NL-A- 8301996	02-01-85
		DE-A- 3473825	13-10-88
		JP-B- 6039386	25-05-94
		JP-A- 60011429	21-01-85
		US-A- 4650677	17-03-87
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